Chapter 4

The influence of boron and allelochemicals released from *Cyperus esculentus* on the growth and development of *Pinus patula* seedlings

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1. Introduction

According to Fisher (1980), a rapidly growing body of data suggests that allelopathy is important in the survival and growth of trees in both plantations and natural stands. The widespread occurrence of woody species that are allelopathic to other species, and sometimes to themselves are extensively researched. Unfortunately very little research has been done on the allelopathic effects of herbaceous angiosperms in forestry.

Fisher, Wood and Glavicic (1978) found that *Solidago* (goldenrod) and *Aster* inhibit the establishment and growth of sugar maple in old fields in Ontario. Horsley (1977) examined regeneration failure in *Prunus serotina* (black cherry) on old fields. He ruled out the effects of browsing, microclimate and competition and concluded that allelopathic inhibition by *Dryopteris novebaracensis* (New York fern) *Brachyelytrum erectum* (shorthusk grass) and *Aster* was the main cause. Poor nutrition and microclimate can be barriers to the establishment of tree species on old fields but allelopathy is often an additional adversity that must be overcome.

Cyperus esculentus is a troublesome weed in many countries, reducing crop yields through competition and allelopathy (Horowitz & Friedman 1971; Tames, Getso & Vieitez, 1973; Stoller, Wax & Slife, 1979 and Drost & Doll, 1980). It can spread asexually by the formation of rhizomes that end in the production of underground tubers, which are recognized as the primary dispersal unit (Wills, Hoagland & Paul, 1980; Stoller & Sweet, 1987; Gifford & Bayer, 1995). According to Stoller, Nema & Bhan (1972) and Thullen & Keeley (1975), tubers may sprout several times before exhausting its energy supply. Therefore, it can be competitive for the entire season.

Boron (B) is an essential micro-element, and the plant's requirements are the greatest during leaf development, flowering and fruit setting. According to Shorrocks (1984), boron deficiency can cause abnormal cell division, i.e., the cell's longitudinal walls remain short and are thus incomplete and irregular. Leaf expansion, distorted leaf development and the lack of internode elongation can also occur. Eventually, shoot and root apical meristems die or become moribund and, after the loss of apical dominance, stunted side shoots develop from the auxiliary meristems. It was reported by Smith & Van Hyussteen (1992) and Schumann & Noble (1993) that the symptoms of poor pine seedling performance on oldlands in South Africa, involved a minimally developed root system, stunting, lack of apical dominance, chlorosis and necrosis of fascicles, and necrosis of the growth tips.

2. Aims

The influence of *C. esculentus* and boron on *P. patula* were evaluated. Specific aims were:

1. To investigate the possibility that boron deficiency can lead to the observed growth problems of *P. patula*
2. To determine the effect of an aqueous *C. esculentus* extract on *P. patula* seedling growth.

3. To measure the effect *C. esculentus* suppression by pine needles have on the growth of *P. patula*.
4. To evaluate the growth of *P. patula* seedlings growing with *C. esculentus* on three types of soil.

3. Materials and Methods

Experiment 1: Effect of added boron, an aqueous *C. esculentus* extract and pine needles on the *P. patula* seedlings grown with, or without, the weed.

Site and growth medium description

The experiment was conducted from October 1997 to December 1998 at the University of Pretoria's experimental farm. The topsoil (0-0.5 m) of two sandy loam soils, afforested and "oldland" soil, were collected in September 1997 at the Giant's Castle Estate of Mondi Forests (Pty) Ltd. KwaZulu-Natal. Afforested soil was collected from a site where no abnormal growth of existing pines was evident. The oldland soil was collected from an adjacent area (approximately 30 m apart), previously used for production of annual crops. Soil properties are given in Table 1.

Table 1 Soil properties of afforested and oldland soil collected in September 1997 at the Giant's Castle Estate of Mondi Forest at Mooi River KwaZulu-Natal

Soil	pH (H_2O)	Bray I P $mgkg^{-1}$	Ca $mgkg^{-1}$	K $mgkg^{-1}$	Mg $mgkg^{-1}$	B $mgkg^{-1}$	C %
Afforested	5.26	2.85	203.3	159	188.5	0.84	3.6
Oldland	4.9	12.54	254.3	276	158.5	1.05	3.7

Upon arrival at the experimental farm, the soils were left in the sun to dry and subsequently sieved. Solid fertilizer in the form of 2:3:2 (24) at the equivalent rate of 200 kg ha^{-1} was added to the soil prior to planting of the pine seedlings. A boron treatment involved addition of boracic acid to oldland soil at the equivalent rate of 10 kg ha^{-1}. Three kilogram of soil was placed into drained plastic pots (195 mm diameter x 160 mm deep) and were kept in a temperature-controlled glasshouse (25°C/ 15°C).

C. esculentus leaf litter were collected from a natural infestation on the oldland. An aqueous extract was prepared by cutting the leaf litter in 20 mm lengths. Fifty grammes of material were mixed with 1000 ml distilled water and macerated in a Waring commercial blender for 30 seconds. The suspension was filtered through Whatman no. 1 filter paper and the supernatant was used to test for allelopathic potential. The extract was kept at 5°C until it was used.

Experimental design

The experiment consisted of four treatments:

1. Afforested soil with no added boron or *C. esculentus* extract (T 1)
2. Oldland soil as described for the afforested soil treatment (T 2)
3. Oldland soil with only boron added (T 3)
4. Oldland soil with added boron and *C. esculentus* leaf litter extract (T 4).

Afforested soil was included in the experiment as it served as a comparison for expected normal seedling growth. *P. patula* seedlings, approximately 80-100 mm in height were obtained from the Mondi Forests nursery at Pietermaritzburg, KwaZulu-Natal. Three days after pots were filled with the soil, a single *P. patula* seedling was transplanted to each pot. Special care was taken not to disturb the root system . Pots received 39% tap water to field capacity, where after watering was done every third day. At each watering, the extract treatment (T 4) received 150 ml water and 100 ml extract. The other treatments received 250 ml tap water. After a week, vigorous leaf development at apical meristems of the pines indicated that they had established successfully, and seedling height and stem diameter were measured. The stem diameter was measured approximately 20 mm from the soil surface. A mark was made on the stem for recurrent diameter measurements. Height was measured from a constant reference point at the soil surface to the apical growing point. Growth parameters were measured fortnightly.

During the first two weeks, herbaceous weeds, especially *C. esculentus* had sprouted on all the oldland soil but not on afforested soil. The *C. esculentus*, was allowed to grow at the three oldland soil treatments, but all other weeds were removed as they emerged. Light competition between the pine seedlings and *C. esculentus* was limited by cutting *C. esculentus* at about 100 mm from the soil surface.

The *C. esculentus* extract was applied for only 14 weeks. Thereafter, all the remaining *C. esculentus* plants were removed from this treatment and subsequently kept weed-free for the rest of the trial period.

Twenty weeks after initial fertilization, 250 ml complete nutrient solution (Nitch, 1972) was administered every third day to all the treatments. Boron was not again added to the treatments.

During September 1998, pine needles collected at the Estate, were placed on the soil surface of treatment T 3 as a groundcover to suppress *C. esculentus* growth.

Harvesting commenced in December 1998. Seedlings were cut at the soil surface and the top-growth placed in a drying oven at 70°C for three days after which the dry mass of the seedlings was determined.

Statistical analysis

Each treatment was replicated ten times. A completely randomized design was used. Analysis of variance was done to determine the effect of the different treatments on the percentage height and stem diameter increase of seedlings. Percentage growth increase was calculated as the final height or stem diameter minus initial height or stem diameter divided by initial height or stem diameter multiplied by 100. Differences between treatment means were identified using the Least Significant Difference test at P=0.05.

<u>Experiment 2</u>: Growth of *P. patula* seedlings on three different types of soil in the presence of *C. esculentus*

Site and growth medium description

The experiment commenced in February 1998 and ended the following February. Three types of soil were collected in December 1997 at the Giant's Castle Estate-soil from a field where no plantation species were cultivated (grassland soil), afforested- and oldland soil. Afforested and oldland soil were collected about 30 m apart. All soils were classified as a sandy loam with soil properties given in Table 2.

Table 2 Soil properties of three types of soil collected in December 1997 at the Giant's Castle Estate

Soil	pH (H_2O)	Bray I P mgkg^{-1}	Ca mgkg^{-1}	Mg mgkg^{-1}	K mgkg^{-1}	B mgkg^{-1}	C %
Grassland	4.66	4.87	122	71	94	0.26	4.6
Afforested	5.23	1.64	171	251	114	0.46	5.2
Oldland	5.3	13.55	245	47	85	0.3	3.9

The soil was left in the sun to dry for three days and then sifted through a 2 mm sieve. Plastic pots, 160 mm in height and 195 mm in diameter with holes in the base for drainage, were filled with 3 kg of each type of soil. The pots were kept in a temperature-controlled glasshouse (25°C/ 15°C) at the experimental farm.

Experimental design

The experiment consisted of soil type as factor (three levels), with or without *C. esculentus*. *P. patula* seedlings, approximately 80-100 mm in height, were obtained from the Mondi Forests nursery at Pietermaritzburg. A day after pots were filled with the soil, a single *P. patula* seedling was transplanted to each pot. Special care was taken so as not disturbed the root system. Approximately 50 g *C. esculentus* tubers collected at the Estate, were planted together with the seedlings. Pots were watered to 39% field capacity with a complete nutrient solution (Nitch, 1972), where after seedlings received a total of 250 ml solution, every second day. *C. esculentus* was cut at intervals to reduce competition for light between the weed and the pine seedling.

Growth parameters were the same as in Exp. 1. Seedlings were harvested twelve months after transplant. They were cut at the soil surface and placed in a drying oven (70°C) for three days where after the dry mass was determined.

Statistical analysis

Each treatment was replicated eight times in a completely randomized design. The same statistical analysis was used for data from both experiments.

4. Results and Discussion

Experiment 1: Effect of added boron, an aqueous *C. esculentus* extract and pine needles on the *P. patula* seedlings grown with, or without, the weed.

In Figure 1 and 2 the height and stem diameter increases of the seedlings over the 14 month growth period is displayed. During the winter, height growth was retarded but during spring and summer, growth increments increased (Figure 1).

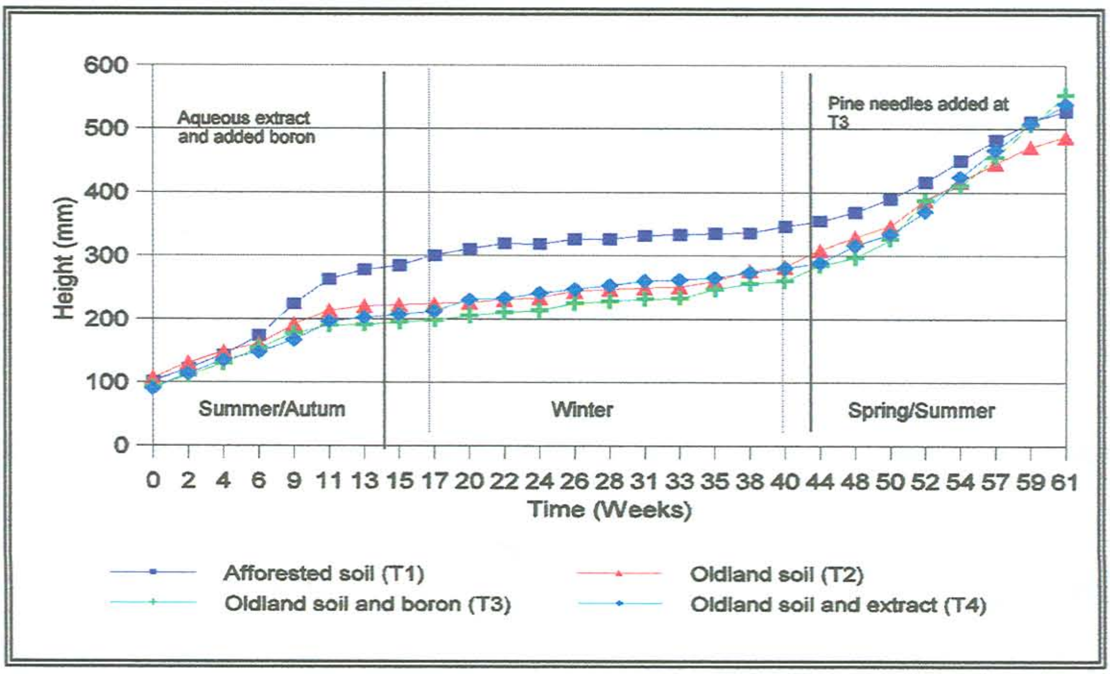

Figure 1 Effect of different treatments on the height growth of *Pinus patula* seedlings during the 14 month growth period

Stem diameter growth is considered to be a more reliable parameter of growth as the stem diameter of the seedlings was not so severely retarded during winter as was observed with height (Figure 2).

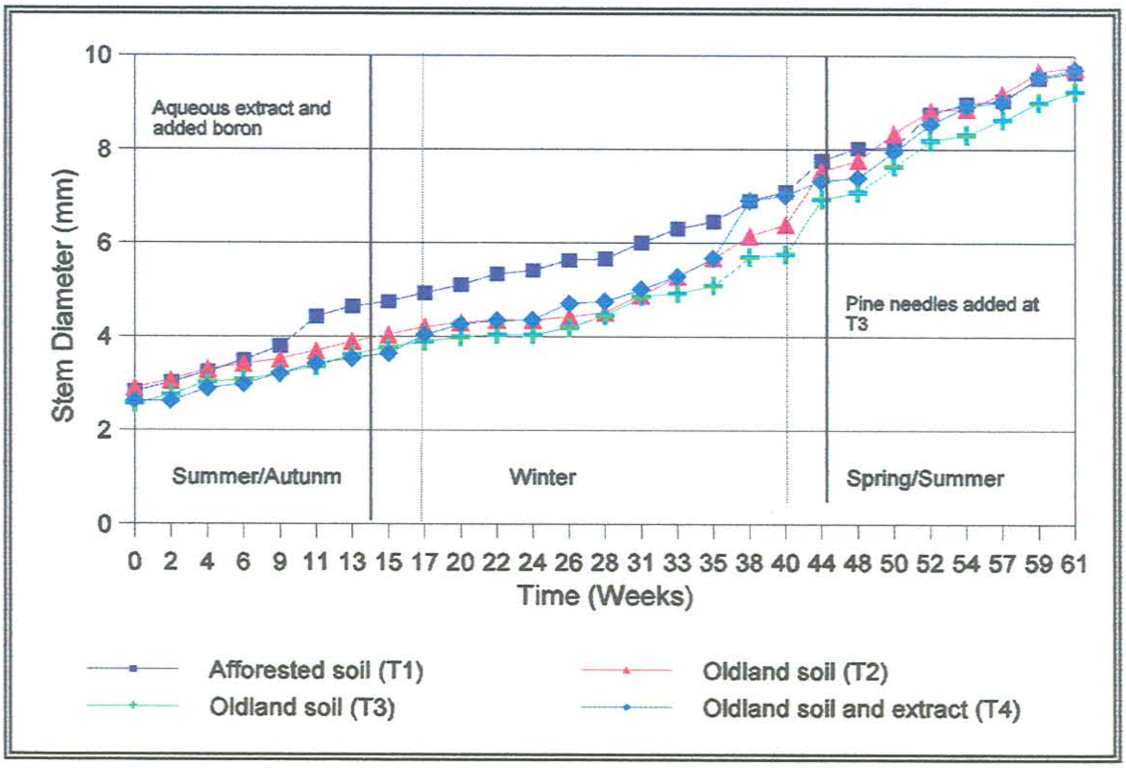

Figure 2 Effect of different treatments on the stem diameter growth of *Pinus patula* seedlings over the 14 month growth period

<u>Experiment 1</u>: Treatment 1: Effect of added boron and an aqueous *C. esculentus* extract on the growth of *P. patula* seedlings over a 14 week period.

Four weeks after being transplanted, seedlings on the oldland soils appeared chlorotic and were noticeably smaller than those on the afforested soil. It is unlikely that these symptoms were due to nutrient deficiencies because all the treatments received the same and adequate nutrition (Table 3).

Table 3 Soil analysis of afforested and oldland soil 14 weeks after the addition of boron and an aqueous *C. esculentus* extract

Treatment	pH (H_2O)	Bray I P $mgkg^{-1}$	Ca $mgkg^{-1}$	K $mgkg^{-1}$	Mg $mgkg^{-1}$	Na $mgkg^{-1}$
Afforested soil	5.22	3.87	505	195	382	45
Oldland soil	5.71	7.67	685	261	430	59
Oldland + Boron	5.77	7.87	721	285	445	60
Oldland +Boron+Extract	5.3	12.42	436	324	310	53

Ten weeks after the chlorotic appearance of the pine seedlings was noticed, the seedlings regained a healthy green colour.

4.1.1 Height

The percentage height increase of seedlings was significantly influenced by the different treatments, 14 weeks after transplant (Table 4). Seedlings growing on afforested soil had a significantly higher height increase than those growing at all three of the oldland soil treatments. This can be attributed to the interference from *C. esculentus* present on the oldland. There were no differences in pine height growth between seedlings growing on the three oldland soil treatments.

Table 4 Effect of added boron and an aqueous *Cyperus esculentus* extract on the percentage height increase of *Pinus patula* seedlings growing in the presence of weeds at 14 weeks after transplant (ANOVA appears in Tables 1, 2 and 3 in Appendix C)

	Final Height (mm)	Initial Height (mm)	Growth Increase (%)
Afforested soil	284.2 a	101.5 a	187.42 a
Oldland soil	222.5 b	106.9 a	112.27 b
Oldland + Boron	194.0 b	92.4 a	112.68 b
Oldland + Boron + Extract	208.5 b	93.0 a	131.10 b
Standard Error	16.01	NS	14.15
CV (%)	13.93		32.94

Means followed by the same letter are not significantly different at P=0.05.

4.1.2 Stem diameter

Seedlings on afforested soil had a significantly higher percentage stem diameter increase than seedlings on oldland soil (Table 5). This result is similar to that for height growth. Stem diameter of pine seedlings in the presence of *C. esculentus* on oldland soils, were reduced by approximately 39% compared to those growing on afforested soil (Table 5). According to Buchanan & Burns (1970), the critical period of weed competition occurs six to eight weeks after agronomic crop emergence. Keeley & Thullen (1975) found *C. esculentus* capable of reducing yields of furrow irrigated cotton when allowed to compete for periods of four weeks or more. Jooste & Van Biljon (1980), reported reduced maize yields from 11.4% to 23.9% with heavy infestations of *C. esculentus*.

Table 5 Effect of added boron and *Cyperus esculentus* extract on the percentage stem diameter increase of *Pinus patula* seedlings growing in the presence of weeds at 14 weeks after transplant (ANOVA appears in Tables 4, 5 and 6 in Appendix C)

	Final Stem Diameter (mm)	Initial Stem Diameter (mm)	Growth Increase (%)
Afforested soil	4.74 a	2.82 ab	69.00 a
Oldland soil	4.00 b	2.92 a	37.55 b
Oldland + Boron	3.76 bc	2.55 b	49.02 b
Oldland + Boron + Extract	3.60 c	2.62 b	38.21 b
Standard Error	0.1	0.09	4.52
CV (%)	8.14	11.26	29.52

Means followed by the same letter are not significantly different at P=0.05.

The applied *C. esculentus* extract had no significant effect on the growth of seedlings. According to Cheng (1992), it is unlikely that extracted allelochemicals from plant material, are those that actually reach the host plant in nature. These chemicals can be transformed during the course of extraction, therefore, allelopathic symptoms may not be manifested at the time or site where plant damage has actually occurred. By the time symptoms are observed, the chemicals may no longer be present. As none of the typical boron deficiency symptoms was evident, and differences between seedlings growing with or without added boron on the oldland soil were not significant, *P. patula* growth abnormalities cannot be ascribed to boron deficiencies in this experiment.

4.2. Experiment 1: Treatment 2: Effect of *P. patula* seedlings growth with or without *C. esculentus* present.

After 14 weeks growth, the extract was no longer applied. *C. esculentus* was removed from this treatment and it was kept weed-free for the rest of the trial period. However, *C. esculentus* was still present on the remaining two oldland soil treatments (T2 and T3).

4.2.1 Height

After eight months, a significant difference in percentage height increase was established between the seedlings growing on afforested soil and seedlings on the oldland soil kept weed-free (Table 6). However, this time seedlings growing on oldland soil had a higher percentage growth increase than seedlings growing on afforested soil. During this time it was winter and relatively no *C. esculentus* were present.

Table 6 Height increase of *Pinus patula* seedlings eight months after *Cyperus esculentus* was removed from one oldland soil treatment but remained in the other two (ANOVA appears in Tables 7, 8 and 9 in Appendix C)

	Final Height (mm)	Initial Height (mm)	Growth Increase (%)
Afforested soil	368.5 a	300.0 a	22.83 b
Oldland soil	328.5 ab	224.0 ab	46.65 a
Oldland soil	296.0 b	198.5 b	49.12 a
Oldland without *C. esculentus*	316.0 b	219.0 b	44.29 a
Standard Error	14.35	10.8	6.06
CV (%)	13.87	14.51	45.78

Means followed by the same letter are not significantly different at P=0.05.

The differences in percentage height increase between seedlings growing at the different oldland treatments were not significant.

4.2.2 Stem diameter

The seedlings growing at all three oldland soil treatments had a significant higher percentage stem diameter increase than seedlings growing on afforested soil (Table 7).

Table 7 Stem diameter increase of *Pinus patula* seedlings eight months after *Cyperus esculentus* was removed from one oldland soil treatment but remained in the other two (ANOVA appears in Tables 10, 11 and 12 in Appendix C)

	Final Stem Diameter (mm)	Initial Stem Diameter (mm)	Growth Increase (%)
Afforested soil	8.02 a	4.91 a	63.71 b
Oldland soil with *C. esculentus*	7.76 ab	4.15 b	86.98 a
Oldland soil with *C. esculentus*	7.07 c	3.88 b	82.45 a
Oldland without *C. esculentus*	7.39 bc	4.04 b	84.00 a
Standard Error	0.2	0.11	4.21
CV (%)	8.44	8.29	16.78

Means followed by the same letter are not significantly different at P=0.05.

Differences in percentage stem diameter increase between seedlings growing on different oldland soils, were not significant. With no herbaceous weed competition, pine seedlings had no interference from *C. esculentus*. William & Warren (1975) suggested that *C. rotundus* must be controlled within three weeks after non-competitive crops emerged, because during the absence of the weed, the crop is likely to suffer little yield reductions.

4.3. Experiment 1: Effect of added pine needles on the growth of *P. patula* seedlings with, or without, the weed

During September 1998, pine needles was placed on the soil surface of the treatment T 3 to suppress *C. esculentus* growth.

4.3.1 Height

The differences in percentage height increase among seedlings growing on afforested and oldland soil, are displayed in Table 8.

Table 8 Height increase of *Pinus patula* seedlings three months after pine needles were placed on the soil surface (ANOVA appears in Tables 12, 13 and 14 of Appendix C)

	Final Height (mm)	Initial Height (mm)	Growth Increase (%)
Afforested soil	554.3 a	386.5 a	36.23 b
Oldland soil	477.3 a	346.0 ab	39.46 b
Oldland + pine needles	552.5 a	325.0 b	70.70 a
Oldland without *C. esculentus*	538.5 a	326.0 b	65.94 a
Standard Error	NS	15.18	5.4
CV (%)		13.87	32.15

Means followed by the same letter are not significantly different at P=0.05.

Pine seedlings growing on oldland soil with *C. esculentus* suppression, had a significantly higher height increase than seedlings growing on afforested soil and oldland soil with weeds. No significant differences in height growth were evident between the treatments with *C. esculentus* suppression and where no weeds were present.

4.3.2 Stem diameter

As with height, the removal or suppression of herbaceous vegetation, resulted in a significant higher percentage stem diameter increase compared to seedlings growing with *C. esculentus* (Table 9). The suppression of *C. esculentus* vegetation with pine needles did not result in a significant increase in the stem diameter of seedlings compared to those growing without the weed. Although no weeds were present on the afforested soil, seedlings had a significant lower percentage stem diameter increase than those on oldland soil without weeds present.

Table 9 Stem diameter increase of *Pinus patula* seedlings after the pine needles was placed on the soil surface (ANOVA appears in Tables 16, 17 and 18 in Appendix C)

	Final Stem Diameter (mm)	Initial Stem Diameter (mm)	Growth Increase (%)
Afforested soil	9.45 a	8.23 a	14.93 b
Oldland soil	9.52 a	8.34 a	14.23 b
Oldland + pine needles	9.25 a	7.48 b	22.52 ab
Oldland without *C. esculentus*	9.55 a	7.59 b	26.09 a
Standard Error	ns	0.2	2.85
CV (%)		8.04	46.28

Means followed by the same letter are not significantly different at P=0.05.

Results obtained are in accordance with those by Nelson, Pedersen, Autry, Dudley & Walstad (1981) and Bacon & Zedaker (1985). In their studies, they concluded that height, stem diameter and biomass of pine seedlings increased when competing vegetation were removed. Schumann, Little & Eccles (1995) concluded that of *Eucalyptus grandis*, *P. patula* and *Acacia mearnsii*, *P. patula* leaf and branch residues had the greatest suppressive effect on the establishment of four weed species. Water extracts of the these residues also resulted in significant suppression of weed establishment, suggesting allelopathic effect.

4.4. Dry mass

The differences in the dry mass of seedlings were not significant (ANOVA appears in Table 19 in Appendix C).

Experiment 2: Growth of *P. patula* seedlings on three types of soil in the presence of *C. esculentus*

1. Height

Percentage height increase was significantly influenced by the different treatments 12 months after transplant. Differences between seedlings growing with or without weeds on grassland or afforested soil, were not significant. Seedlings growing without weeds on the oldland soil had a significant greater height increase than seedlings with weeds on both grassland - and oldland soil (Figure 3). This could be due to the absence of *C. esculentus*.

Seedlings on oldland soil without weeds, showed the highest percentage height increase (182.74%). A significant difference was obtained between the percentage height increase of seedlings on oldland soil and grassland soil both without weeds. Height differences between height of seedlings growing with weeds on the different soils, were not significant.

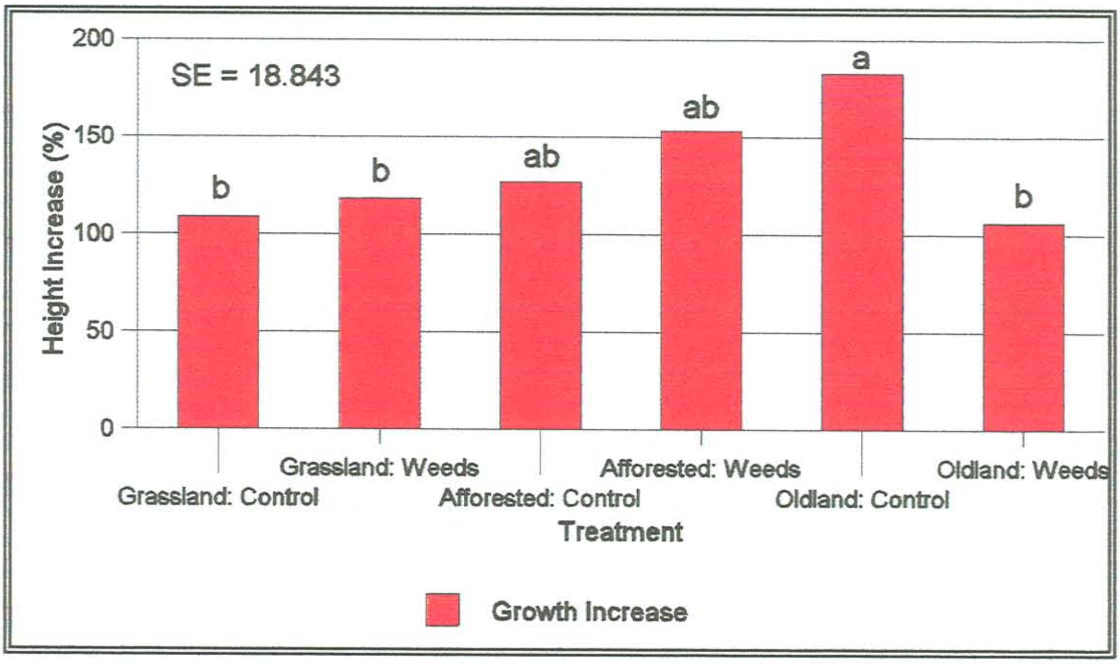

Figure 3 Effect of three types of soil with or without *Cyperus esculentus* on the percentage height increase of *Pinus patula* seedlings (ANOVA appears in Tables 20, 21 and 22 in Appendix C)

2. Stem diameter

The percentage stem diameter increase between seedlings at the different treatments was not significant (ANOVA appears in Tables 23, 24 and 25 in Appendix C).

3. Dry mass

Differences between the dry mass of seedlings at the different treatments were not significant (ANOVA appears in Table 26 in Appendix C).

5. Conclusions

The significant negative response of *P. patula* seedlings in the presence of *C. esculentus*, indicates the existence of an underlying biotic component in the poor growth of seedlings on oldland soil. *C. esculentus* was actively growing when the soil was collected and therefore the concentration of allelochemicals in the soil would have been considerably higher when the weed was actively growing with the seedlings, having a more inhibitory effect on seedling growth. Partial recovery from the detrimental effects of these compounds was observed during the winter when no weeds were present. In a similar fashion, seedlings had a significant higher growth increase when the growth of *C. esculentus* was suppressed by the addition of pine needles as a groundcover, or when the weed was removed. The results obtained from the experiment also indicate that boron deficiency is probably not responsible for abnormal growth of tree seedlings on oldland soil, as none of the deficiency symptoms were observed. Results suggests that *P. patula* seedlings can be successfully established on oldland soil, provided that *C. esculentus* control is practiced.

6. References

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Appendix C

1. Added boron and aqueous *Cyperus esculentus* extract

Table 1 Effect of added boron and an aqueous *Cyperus esculentus* extract on the beginning height of *Pinus patula* seedlings 14 weeks after transplant

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	3	1470.100	490.033	1.34	0.2762
Error	36	13149.800	365.272		
Total	39	14619.900			

$R^2 = 0.1006$

Table 2 Effect of added boron and an aqueous *Cyperus esculentus* extract on the final height of *Pinus patula* seedlings 14 weeks after transplant

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	3	47229.800	14743.267	15.70	0.0001
Error	36	36092.600	1002.572		
Total	39	83322.400			

$R^2 = 0.5668$

Table 3 Effect of added boron and an aqueous *Cyperus esculentus* extract on the percentage height increase of *Pinus patula* seedlings 14 weeks after transplant

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	3	37755.547	12585.182	6.28	0.0015
Error	36	72121.036	2003.362		
Total	39	109876.583			

$R^2 = 0.3436$

Table 4 Effects of added boron and *Cyperus esculentus* on the beginning stem diameter of *Pinus patula* seedlings 14 weeks after transplant

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	3	0.887	0.296	3.14	0.0372
Error	36	3.393	0.094		
Total	39	4.280			

$R^2 = 0.2072$

Table 5 Effects of added boron and *Cyperus esculentus* on the final stem diameter of *Pinus patula* seedlings 14 weeks after transplant

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	3	7.627	2.542	23.66	0.0001
Error	36	3.868	0.107		
Total	39	11.495			

$R^2 = 0.6635$

Table 6 Effects of added boron and *Cyperus esculentus* on the percentage stem diameter increase of *Pinus patula* seedlings 14 weeks after transplant

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	3	6488.235	2162.745	10.57	0.0001
Error	36	7368.013	204.667		
Total	39	13856.248			

$R^2 = 0.4683$

2. Treatment kept weed-free

Table 7 Beginning height of *Pinus patula* seedlings eight months after the aqueous *Cyperus esculentus* extract was no longer applied and the treatment kept weed-free

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	3	59336.875	19778.958	16.96	0.0001
Error	36	41982.500	1166.181		
Total	39	101319.375			

$R^2 = 0.5856$

Table 8 Final height of *Pinus patula* seedlings eight months after the aqueous *Cyperus esculentus* extract was no longer applied and the treatment kept weed-free

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	3	28062.500	9354.167	4.54	0.0084
Error	36	74135.000	2059.306		
Total	39	102197.500			

$R^2 = 0.2746$

Table 9 Percentage height increase of *Pinus patula* seedlings eight months after the aqueous *Cyperus esculentus* extract was no longer applied and the treatment kept weed-free

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	3	4563.131	1521.044	4.14	0.0127
Error	36	13217.112	367.142		
Total	39	17780.243			

$R^2 = 0.2566$

Table 10 Beginning stem diameter of *Pinus patula* seedlings eight months after the aqueous *Cyperus esculentus* extract was no longer applied and the treatment kept weed-free

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	3	6.265	2.088	16.88	0.0001
Error	36	4.454	0.124		
Total	39	10.719			

$R^2 = 0.5845$

Table 11 Final stem diameter of *Pinus patula* seedlings eight months after the aqueous *Cyperus esculentus* extract was no longer applied and the treatment kept weed-free

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	3	5.206	1.735	4.26	0.0113
Error	36	14.670	0.408		
Total	39	19.876			

$R^2 = 0.2619$

Table 12 Percentage stem diameter increase of *Pinus patula* seedlings eight months after the aqueous *Cyperus esculentus* extract was no longer applied and the treatment kept weed-free

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	3	3338.632	1112.877	6.29	0.0015
Error	36	6373.236	177.034		
Total	39	9711.868			

$R^2 = 0.3438$

3. Pine needles as groundcover

Table 13 Beginning height of *Pinus patula* seedlings two months after pine needles was placed on the soil surface of one of the treatments

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	3	24815.000	8271.667	3.59	0.0228
Error	36	82945.000	2304.028		
Total	39	107760.000			

$R^2 = 0.2303$

Table 14 Final height of *Pinus patula* seedlings two months after pine needles was placed on the soil surface of one of the treatments

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	3	39445.900	13148.633	1.44	0.2471
Error	36	328615.200	9128.200		
Total	39	368061.100			

$R^2 = 0.1072$

Table 15 Percentage height increase of *Pinus patula* seedlings two months after pine needles was placed on the soil surface of one of the treatments

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	3	9453.414	3151.138	10.82	0.0001
Error	36	10485.623	291.268		
Total	39	19939.067			

$R^2 = 0.4741$

Table 16 Beginning stem diameter of *Pinus patula* seedlings two months after the pine needles was placed on the soil surface

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	3	5.746	1.914	4.74	0.0069
Error	36	14.550	0.404		
Total	39	20.296			

$R^2 = 0.2831$

Table 17 Final stem diameter of *Pinus patula* seedlings two months after the pine needles was placed on the soil surface

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	3	1.007	0.336	0.42	0.7376
Error	36	28.551	0.793		
Total	39	29.558			

$R^2 = 0.0340$

Table 18 Percentage stem diameter increase of *Pinus patula* seedlings two months after the pine needles was placed on the soil surface

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	3	1010.589	336.953	4.16	0.0125
Error	36	2914.754	80.965		
Total	39	3925.613			

$R^2 = 0.2575$

Table 19 Dry mass of *Pinus patula* seedlings 14 months after the seedlings were transplanted

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	3	89.156	29.719	0.71	0.5530
Error	36	1508.922	41.914		
Total	39	1598.078			

$R^2 = 0.0558$

4. Growth on three types of soil

Table 20 Effect of three types of soil with or without *Cyperus esculentus* on the beginning height of *Pinus patula* seedlings

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	5	4872.917	974.583	3.28	0.0137
Error	42	12475.000	297.034		
Total	47	17347.917			

$R^2 = 0.2809$

Table 21 Effect of three types of soil with or without *Cyperus esculentus* on the final height of *Pinus patula* seedlings

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	5	47040.104	9408.021	1.47	0.2205
Error	42	269084.375	6406.771		
Total	47	316124.479			

R^2 = 0.1488

Table 22 Effect of three types of soil with or without *Cyperus esculentus* on the percentage height increase of *Pinus patula* seedlings

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	5	35593.688	7118.738	2.51	0.0450
Error	42	119297.021	2840.405		
Total	47	154890.709			

R^2 = 0.2298

Table 23 Effect of three types of soil with or without *Cyperus esculentus* on the beginning stem diameter of *Pinus patula* seedlings

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	5	0.309	0.062	1.55	0.1964
Error	42	1.676	0.040		
Total	47	1.985			

R^2 = 0.1554

Table 24 Effect of three types of soil with or without *Cyperus esculentus* on the final stem diameter of *Pinus patula* seedlings

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	5	9.408	1.882	0.67	0.6517
Error	42	118.745	2.827		
Total	47	128.153			

R^2 = 0.0734

Table 25 Effect of three types of soil with or without *Cyperus esculentus* on the percentage stem diameter increase of *Pinus patula* seedlings

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	5	54498.523	10899.705	1.67	0.1632
Error	42	274260.824	6530.020		
Total	47	328759.347			

R^2 = 0.1658

Table 26 Dry mass of *Pinus patula* seedlings 12 months after the seedlings were transplanted

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	5	621.290	124.258	1.56	0.1934
Error	42	3353.511	79.846		
Total	47	3974.812			

R^2 = 0.1563